PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of	:	
Gholam A. PEYMAN	:	Appeal No. _____
Serial No.: 10/073,863	:	Group Art Unit: 1615
Filed: February 14, 2002	:	Examiner: H. Sheikh
For: METHOD AND COMPOSITION FOR HYPERTHERMALLY TREATING CELLS	:	

BRIEF ON APPEAL

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TABLE OF CONTENTS

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42561

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

For the Appeal to the Board of Patent Appeals and Interferences from the decision of March 21, 2005 finally rejecting claims 1, 4-10, 13-27, 32 and 34-40, Applicant submits the following Brief on Appeal in accordance with 37 C.F.R. § 1.192.

I. Real Party in Interest

The real party in interest in this application is the applicant, Gholam A. Peyman.

II. Related Appeals and Interferences

There are no related applications on appeal or involved in an interference.

III. Status of the Claims

Claims 1, 4-10, 13-32 and 34-40 are pending and on appeal. Claims 2, 3, 11, 12 and 33 are cancelled. Claims 28-31 are allowed.

IV. Status of the Amendments

An amendment after the final rejection was filed to correct a minor clerical error to claim 28. This amendment is indicated as being entered upon filing of the Notice of Appeal.

V. Summary of the Invention

The present invention is directed to a method of hyperthermally treating animal tissue and cells in a target site by heating to a temperature and for a sufficient time to hyperthermally treat and kill cells in the target site as disclosed in paragraph 0027. The invention is specifically directed to methods of killing cells in the target site by heating to a sufficient temperature below the protein denaturization temperature and substantially without denaturing the proteins. Since hyperthermally treating cells requires that a minimum temperature be attained, the invention introduces a temperature sensitive indicator to indicate that a predetermined temperature is attained sufficient to hyperthermally treat the cells in the tissue. Paragraph 0029.

During the heating of the tissue, the heat is absorbed by the tissue and body fluids so that the temperature of the tissue cannot be determined by visual inspection. Thus, the physician or technician can unknowingly exceed the protein denaturization temperature. In order to detect

when a minimum or threshold temperature is attained, the invention introduces a fluorescent dye encapsulated in a liposome into the target site. The liposome is selected to release the dye when a selected temperature is attained sufficient to kill cells, and thus, attain the hyperthermal treatment. The dye is fluoresced and visualized in the target site where the heat is applied to provide a visual indication that a predetermined temperature is attained. The temperature of the tissue cannot be visually determined without the use of the fluorescent dye and cannot be determined by other conventional means. Paragraph 0029.

Protein denaturization begins to occur at 50°C and occurs rapidly at 60°C. Paragraph 0032. Therefore, it is desirable to maintain the temperature below 60°C, and preferably less than 50°C.

The invention of claim 1 is directed to a method of hyperthermally treating tissue for sufficient time to kill cells without denaturing proteins. Thus, the method of the invention provides a visual temperature detection and controls the hyperthermal treatment within a temperature range sufficient to kill the cells without excessive heating that can cause denaturing of proteins. In one embodiment, a heat sensitive liposome encapsulating a fluorescent dye is introduced into the bloodstream to flow through the target site. The liposome is selected to release the dye at a temperature of about 45°C to 49°C. A heat source is then applied to hyperthermally heat the tissue in the target site to at least 47°C for a time sufficient to kill cells without denaturing the proteins, release the dye, and visualize the dye to indicate that a temperature of at least 45°C has been attained which is sufficient to hyperthermally treat the tissue. Paragraph 0033.

In another embodiment of the invention recited in claim 10, the invention is directed to a method of detecting a threshold temperature and hyperthermally treating tissue. The method

introduces a first fluorescent dye encapsulated in a first heat sensitive liposome into the bloodstream to flow through a target site. The first liposome is selected to release the dye at a temperature of 45°C to 49°C. The target site is heated to a temperature to release the first fluorescent dye and the dye is fluoresced to visualize a tissue temperature when the temperature of the tissue reaches at least 45°C. The heating is continued for a time sufficient to hyperthermally treat the tissue and kill cells at a temperature below the protein denaturization temperature. A second liposome containing a second dye is introduced to release the second dye at a temperature of at least 50°C. The second dye provides an indicator that a maximum desired temperature has been attained so that when the second dye is visualized, the technician can reduce the temperature of the tissue to a temperature of less than 50°C. In other embodiments of the invention, the target site is heated to a temperature of about 47°C to 49°C and the second liposomes are selected to release the second fluorescent dye at a temperature of about 50°C to 60°C, thereby providing an indication that the temperature should be reduced.

VI. Grounds of Rejection to be Reviewed on Appeal

The issue for review on appeal is:

1. Whether claims 1, 4-10, 13-27, 32 and 34-40 directed to a method of hyperthermally treating tissue to kill cells and releasing and fluorescing a dye to indicate a temperature sufficient to hyperthermally treat the tissue is obvious under 35 U.S.C. § 103(a) over U.S. Patent No. 5,935,942 to Zeimer disclosing a method of visualizing vasculature in view of U.S. Patent No. 5,976,502 to Khoobehi et al. disclosing the use of two dyes in visualizing blood flow and U.S. Patent No. 3,993,754 to Rahman disclosing a liposome encapsulated drug where

none of the cited patents attain a temperature to hyperthermally treat tissue or use the liposomes as a temperature indicator.

VII. Grouping of the Claims

The claims do not stand or fall together. Each of the claims are separately patentable for the reasons discussed herein.

VIII. Argument

Claims 1, 4-10, 13-27, 32 and 34-40 are rejected as being obvious over U.S. Patent No. 5,935,942 to Zeimer in view of U.S. Patent No. 5,976,502 to Khoobehi et al. and further in view of U.S. Patent No. 3,993,754 to Rahman. The rejection is based on the position that the cited art discloses heating tissue and introducing liposomes into the body. The rejection alleges that it would be obvious to modify the heating step of the primary reference according to the secondary references. For the reasons discussed herein, the proposed combination of the cited patents does not render the claimed invention obvious. The cited patents as a whole do not disclose 1) thermally treating tissue to kill cells, 2) detecting a temperature sufficient to hyperthermally treat tissue, 3) releasing and fluorescing a fluorescent dye when the threshold temperature is attained, or 4) releasing and fluorescing a fluorescent dye when an upper limit is attained to provide an indication to the technician to reduce the temperature.

The rejection appears to disregard the plain language of the claims for those portions of the claims that are not found in the cited art. Other portions of the claimed invention that are not found in the art of record are alleged to be "not critical", although no reasonable basis for this

position is provided. The cited patents do not disclose or suggest the claimed invention when viewed as a whole.

Page 6 of the final rejection states that the claimed temperature range of 45° to 60° is not critical and that the claims "merely require that the tissue be heated for a sufficient time to kill cells". However, this feature of the claimed invention is clearly not disclosed or suggested in the art of record. It is improper to disregard portions of the claimed language that cannot be found in the cited art. The claims do not "merely" require heating the cells as suggested in the Action. Claim 1 recites a specific temperature of 45°C to 60°C to thermally treat the tissue and kill the cells.

The Advisory Action asserts that the claimed temperature of 45°C does not define over the prior patents disclosing a temperature of 40°C although no basis for this position is presented. However, the difference between 40°C and 45°C when treating tissue in the body is important. A temperature of 40°C is below the threshold temperature and will not hyperthermally treat the tissue, while a temperature of 45°C is effective. Thus, the difference between the claimed temperature and the temperature of the cited patents is not insignificant.

A. <u>The Cited Patents Do Not Disclose Hyperthermal Heating</u>

The cited patents do not disclose hyperthermally treating tissue. The Zeimer patent is cited in the final rejection for disclosing a method of chemically treating tissue and cells. The final rejection recognizes that Zeimer discloses releasing a chemical component to cause tissue damage and occlusion of blood vessels. Notwithstanding the disclosed method of Zeimer, the Action concludes that some thermal damage occurs in the process of Zeimer. However, the

Action does not provide any reasonable basis for this position, which is inconsistent with the disclosure of Zeimer.

Zeimer and the secondary patents do not disclose or suggest hyperthermally treating tissue as in the claimed invention. Furthermore, the cited patents do not disclose or suggest heating tissue to a temperature sufficient to kill cells at a temperature below the denaturization temperature of the proteins and do not disclose hyperthermally treating tissue to a temperature of at least 47°C as in claim 1.

As disclosed in column 5, lines 51-54 of Zeimer, the invention of Zeimer is directed to a method of chemically occluding blood vessels. Zeimer defines "chemically occluding" in column 6, lines 1-2 as being a non-thermal means of occluding where the chemical occlusion is the result of a chemical, biological, pharmaceutical or pharmacological agent that causes physiological and/or structural damage to vasculature. Thus, Zeimer is expressly directed to chemical occlusion and not to thermal treatment of the tissue.

As disclosed in column 7, lines 45-64, Zeimer releases the chemical agent from liposomes by non-invasively heating the tissue. As defined in this passage, non-invasively heating means heating without causing substantial damage to the tissue. To prevent the damage to the tissue, the non-invasive heating step of Zeimer heats the blood vessel to approximately 41°C. The temperature of 41°C releases the contents of the liposome into the vasculature of the tissue without causing substantial damage to the vasculature while minimizing heating of extra vascular tissue. Thus, Zeimer specifically discloses that heating to a temperature of 41°C does not thermally treat the tissue or cells. Notwithstanding this disclosure, the Examiner suggests that Zeimer hyperthermally treats the tissue and that there is no significant difference between the temperature of Zeimer.

Page 7 of the final rejection recognizes that Zeimer discloses heating to 41°C without causing substantial physiological damage. However, the final rejection then concludes that this indicates that some tissue damage occurs. This position is not supported by facts or evidence of record. Moreover, this statement is contrary to the specific teachings of Zeimer. Thus, the final rejection is incorrect in asserting that "some tissue damage does occur" in the process of Zeimer.

On page 9 of the final rejection, the rejection also relies on the contention that "the prior art does teach the generic concept of using temperature indicating means to effectively chemically treat tissue" supports the rejection. However, this statement mischaracterizes the invention as claimed and mischaracterizes the cited art. Zeimer and Khoobehi et al. disclose liposomes as temperature-sensitive carriers to release the dye and/or a chemical agent. The liposomes of Zeimer and Khoobehi et al. are not temperature indicating means as suggested in the Action. Furthermore, the Action fails to establish the prima facie obviousness of the claimed invention of thermally treating tissue and killing cells where the cited art only discloses chemically treating tissue.

For the reasons discussed above, Zeimer does not disclose or suggest applying a heat source to a target site and hyperthermally treating the tissue for a sufficient time to kill cells in the tissue substantially without denaturing proteins in the tissue. Furthermore, Zeimer discloses heating the tissue to avoid cell damage by heating to a temperature of 41°C. As recognized in the final rejection, Zeimer does not disclose heating the tissue to a temperature of at least 47°C as recited in claim 1. However, the final rejection suggests that the difference between the 41°C of Zeimer and the claimed temperature of at least 47°C is insignificant. Heating tissue to a temperature of 47°C is selected to cause tissue damage and kill the cells in the target site. In contrast, the temperature of 41°C of Zeimer does not cause tissue damage, does not kill the cells,

and is specifically selected by Zeimer to avoid tissue damage. Therefore, the claimed temperature of at least 47°C is an important aspect of the invention that is not disclosed or suggested in Zeimer or the secondary patents. The rejection provides no basis for the position that the claimed temperature is obvious over the temperature disclosed by Zeimer. A temperature difference of 6°C when treating cells and tissue in the body is indeed significant and cannot be ignored.

Page 7 of the final rejection suggests that the claims "merely require that the tissue be heated for a sufficient time to kill cells". This statement is incorrect and mischaracterizes the claimed invention. Claim 1 specifically recites heating the tissue to at least 47°C to kill the cells in the target site and treating the cells substantially without denaturing proteins in the tissue. These claimed features appear to be disregarded in the rejection.

Khoobehi et al. is cited for disclosing a method of observing blood flow through the eye by injecting a carrier such as liposomes into the bloodstream to visualize particles flowing through the blood vessels. One embodiment of Khoobehi et al. introduces two particulate carriers containing different fluorescent dyes so that the two dyes can be selectively fluoresced while encapsulated in the particles by subjecting to a suitable laser to fluoresce one or both dyes. Khoobehi et al. does not disclose or suggest treating tissue either chemically or thermally and does not heat to intentionally release the dye. Khoobehi et al. does not disclose or suggest hyperthermally treating tissue, killing cells in the target site, heating the tissue to a temperature of at least 47°C, or releasing the dye to provide an indication that a threshold temperature has been attained. Accordingly, Khoobehi et al. fails to provide the deficiencies of Zeimer.

Rahman is cited for disclosing liposomes containing a drug such as a cancer treatment drug. Rahman does not disclose hyperthermally treating tissue, and thus, does not provide the deficiencies of Zeimer or Khoobehi et al.

B. The Cited Patents Do Not Disclose Detecting a Threshold Temperature to Hyperthermally Treat Cells

The cited patents neither standing alone or in combination also do not disclose or suggest detecting a threshold temperature when a heat source is applied to the target site. Moreover, the cited patents do not disclose or suggest applying a heat source to the target site to hyperthermally heat the tissue to a temperature of at least 47°C to release a fluorescent dye contained within a liposome that ruptures at 45-49°C and visualizing the dye to indicate that a temperature has been attained as in claims 1, 10 and 23. Zeimer discloses that the liposomes containing the chemical treating agent can contain a fluorescent dye which can be fluoresced when released. Zeimer releases the dye to visualize the blood flow through the blood vessels. Zeimer does not disclose or suggest detecting the dye as a method of determining a minimum temperature for treating the tissue. Furthermore, Zeimer provides no suggestion of heating to 47°C and detecting a temperature sufficient to release a dye from a liposome that releases the dye at a temperature of at least 45°C.

Khoobehi et al. relates to a method where the liposomes contain a fluorescent dye so that the liposomes can be detected as particles by fluorescing the dye within the liposomes. Khoobehi et al. does not disclose or suggest releasing a dye and fluorescing the dye by heating to a temperature sufficient to hyperthermally treat and kill cells and fluorescing the dye to indicate that a minimum temperature for treating the cells has been attained. Rahman also fails to

disclose releasing a fluorescent dye from liposomes and fluorescing the dye to indicate that a minimum or threshold temperature has been attained. Accordingly, the combination of these cited patents do not disclose these features of the claimed invention as recited in independent claims 1, 10 and 23.

The combination of Zeimer, Khoobehi et al. and Rahman do not disclose or suggest the method of claim 1 since the patents do not disclose introducing liposomes containing a fluorescent dye into the bloodstream where the liposomes release the dye at a temperature of about 45°C to about 49°C, hyperthermally treating the tissue to at least 47°C to hyperthermally treat the tissue and kill the cells in the tissue without denaturing the proteins in the tissue or fluorescing and visualizing the dye to indicate that a predetermined temperature has been attained at the target site.

On page 5 of the final rejection, the Examiner recognizes that Zeimer does not teach hyperthermally treating tissue to kill cells. Claim 1 specifically recites heating the tissue for a sufficient time to kill the cells which is one feature of the invention which is not disclosed in the art of record. Furthermore, the claimed temperature of the release of the dye from the liposomes of at least 45°C provides a specific indication that a temperature is attained sufficient to kill the cells. The temperature of Zeimer limits the temperature at which the liposomes are ruptured to 41°C specifically to avoid thermal damage to the tissue. In contrast, the claimed invention is specifically directed to damaging the tissue by hyperthermally treating.

The Examiner provides no basis for the position that it is obvious to modify Zeimer to increase the temperature well above the maximum temperature provided by Zeimer to attain a result that is specifically avoided by Zeimer, Khoobehi et al. and Rahman. The Examiner has not established prima facie obviousness to hyperthermally treat tissue and kill cells by heating to

a temperature of at least 47°C as in claim 1 where the art of record specifically limits the temperature to avoid damage to the cells. Accordingly, independent claim 1 is not obvious over the combination of Zeimer, Khoobehi et al. and Rahman.

Independent claim 10 is directed to a method of detecting a threshold temperature and hyperthermally treating tissue by introducing a first fluorescent dye encapsulated in a liposome into the bloodstream where the first liposome releases the dye at a temperature of 45-49°C and heating the target site to release the dye and indicate and visualize a tissue temperature when the tissue reaches at least 45°C, and thereafter continuing heating the target site to hyperthermally treat the tissue and kill cells in the tissue at a temperature below the protein denaturing temperature. As discussed above, Zeimer does not disclose or suggest a liposome that releases a fluorescent dye at a temperature of 45-49°C and does not heat the tissue to a temperature of at least 45°C. Moreover, Zeimer does not heat the target site to release the fluorescent dye and fluoresce the dye to indicate and visualize a temperature of at least 45°C. Khoobehi et al. and Rahman also fail to disclose these method steps. Accordingly, the combination of these cited patents does not render claim 10 obvious to one of ordinary skill the art.

Independent claim 23 is directed to a method of hyperthermally treating tissue by introducing a temperature indicating substance in the form of a fluorescent dye encapsulated in a liposome where the dye is released at a temperature of 45-49°C and introducing a second fluorescent dye encapsulated in a second liposome where the second dye is released at a temperature of at least 50°C. Claim 23 further recites heating the target site to a temperature to release the first dye from the first liposome and fluorescing the dye to indicate an effective temperature of at least 45°C for hyperthermally treating the tissue without releasing the second dye from the second liposomes.

Khoobehi et al. is cited for disclosing the use of two liposomes containing different fluorescent dyes. However, Khoobehi et al. does not disclose introducing that rupture at a temperature above the temperature at which cell damages occurs as in the claimed invention. Khoobehi et al. also fail to disclose heating a target site to a temperature of at least 45°C to release the first dye without releasing the second fluorescent dye from the second liposome. Therefore, Khoobehi et al. does not provide the deficiencies of Zeimer. The combination of Zeimer, Khoobehi et al. and Rahman do not render independent claim 23 obvious to one of ordinary skill in the art.

For the reasons discussed above, independent claims 1, 10 and 23 are not obvious over the combination of Zeimer, Khoobehi et al. and Rahman.

C. <u>The Dependent Claims are Not Obvious</u>

The dependent claims are also not obvious over the cited art for disclosing features of the invention that are not disclosed or suggested in the art of record in combination with the features of the independent claims. For example, the combination of the cited art does not disclose the bioactive compound of claims 4, 5, 6 and 7, or the heat source of claims 8 and 9, in combination with the liposomes that release the dye at a temperature of 45-49°C or heating the tissue to a temperature of at least 47°C to release the dye of claim 1.

Claim 13 depends from claim 10 to recite the step of heating the target site to a temperature of 47-49°C for about 1 to 10 minutes. For the reasons discussed above, Zeimer specifically limits the temperature of the tissue to 41°C to prevent damage to the tissue. In contrast, the claimed invention specifically heats the tissue to a temperature to cause damage and kill the cells. Heating the tissue to 47°C as in claim 13 provides rapid cell damage.

Accordingly, it is not obvious to one or ordinary skill in the art to modify Zeimer as suggested in the Action to heat the tissue to a temperature of 47°C to 49°C as in claim 13. Thus, claim 13 is not obvious over the combination of the cited patents.

The cited art does not disclose or suggest the bioactive compound of claims 14-17 encapsulated within a liposome or the heat source of claims 18 and 19 in combination with the method steps of claim 10.

Claim 20 depends from claim 10 to recite the step of introducing a second fluorescent dye encapsulating the second liposome where the second liposome releases the dye at a temperature of at least 50°C and visualizing detecting the second fluorescent dye released from the second liposome and thereafter reducing the temperature of the tissue to a temperature below 50°C in response to the detected second dye. Zeimer, Khoobehi et al. and Rahman provide no suggestion to introduce a second liposome that ruptures or releases a fluorescent dye at a temperature of 50°C. The art of record also clearly fails to disclose the step of visualizing and detecting the second fluorescent dye released from the second liposome and thereafter reducing the temperature to a temperature below 50°C in response to the detected second dye. The art of record provides no motivation or incentive to one of ordinary skill in the art to carry out these claimed steps either alone or in combination with the method steps of claim 10. Accordingly, claim 20 is not obvious to one of ordinary skill in the art.

Claim 21 depends from claim 20 to recite that the second fluorescent dye is released from the second liposome at a temperature where protein denaturization occurs and reducing the temperature below the protein denaturization temperature in response to the detected second fluorescent dye. Claim 22 depends from claim 21 to recite the step of heating the tissue below the protein denaturization temperature and below the temperature at which the second dye is

released. These steps are not disclosed or suggested in the cited art. Accordingly, these claims are allowable over the art of record.

Claim 24 depends from claim 23 to recite the step of monitoring and detecting the second fluorescent dye when released from the second liposome and reducing the temperature below the protein denaturing temperature. For the reasons discussed above, the art of record does not disclose reducing the temperature of the tissue in response to a detected dye as in claim 24 or the second dye being a different color from the first dye as in claim 25. The art of record also fails to disclose the phospholipids which form the liposomes of claim 26, the bioactive compound of claims 30 and 31, heating the tissue to a temperature of 47-49°C as in claim 32, a second temperature sensitive liposome releasing the dye at a temperature of 50-60°C as in claim 34, the target site being in the eye as in claim 38, heating the target site to a temperature below the temperature where the second dye is released as in claim 39, or detecting the second fluorescent dye and thereafter reducing the temperature of the tissue when the second dye is detected as in claim 40 in combination with the features of independent claim 23. Accordingly, these claims are allowable over the art of record.

IX. Conclusion

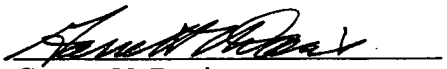
The final rejection does not establish prima facie obviousness of the invention as claimed. Each of the independent claims are directed to a method of hyperthermally treating tissue and killing cells. Thus, the claimed invention is specifically directed to damaging the cells by heating to a temperature above the temperature at which the cells are killed without denaturing the proteins in the tissue. Zeimer, Khoobehi et al. and Rahman specifically disclose heating to a temperature below the temperature at which cell damage occurs. Thus, the cited patents

specifically avoid damaging the tissue and the cells. It is not obvious to one of ordinary skill in the art to modify Zeimer in a manner that will destroy the intended function of Zeimer, namely heating to prevent tissue damage. Zeimer specifically discloses heating to a maximum temperature of 41°C to avoid tissue damage and provide a non-invasive heating step. In contrast, the claimed invention specifically recites heating the tissue to a temperature of at least 47°C to thereby hyperthermally treat the tissue and kill cells in a manner that is otherwise avoided by Zeimer.

Zeimer, Khoobehi et al. and Rahman either standing alone or in combination provide no motivation or incentive to introduce a liposome containing a fluorescent dye into the bloodstream and releasing the dye and fluorescing the dye as an indication that a threshold temperature has been attained in order to hyperthermally treat the tissue and kill cells in the target site. Accordingly, the claims are allowable over the art of record.

In view of the deficiencies of the cited patents, the claims are not obvious to one of ordinary skill in the art. Accordingly, reversal of the final rejection is requested.

Respectfully submitted,



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Dated: Oct 21, 2005

APPENDIX - Listing of Claims on Appeal

1. (Previously presented) A method of hyperthermally treating tissue in an animal, said method comprising the steps of:

introducing a temperature indicating substance into the bloodstream of said animal to flow through said tissue in a target site, said temperature indicating substance including a fluorescent dye encapsulated within a heat sensitive liposome, said fluorescent dye being releasable from said liposome at a temperature of about 45°C to about 49°C, and

applying a heat source to said target site and hyperthermally heating said tissue in said target site to at least 47°C to release said dye and to hyperthermally treat said tissue in said target site for a time sufficient to kill cells in said tissue substantially without denaturing proteins in said tissue, and fluorescing and visualizing said dye to indicate that a predetermined temperature has been attained at said target site.

Claim 2. (Cancelled)

Claim 3. (Cancelled)

4. (Previously presented) The method of claim 1, wherein said liposome encapsulates a bioactive compound, and said method comprises heating said liposome to release said bioactive compound.

5. (Previously presented) The method of claim 4, wherein said bioactive compound is heat activated.

6. (Original) The method of claim 4, wherein said bioactive compound is an antiproliferative agent or an antitumor agent.

7. (Previously presented) The method of claim 4, wherein said bioactive compound is selected from the group consisting of cisplatin, carboplatin, tetraplatin, iproplatin, adriamycin, mitomycin C, actinomycin, ansamitocin and bleomycin.

8. (Original) The method of claim 1, wherein said heat source is a laser source, a microwave source, an infrared source, or an ultrasonic source.

9. (Original) The method of claim 1, wherein said heat source is a heated fluid source, and where said method comprises applying said heated fluid to said target site.

10. (Previously presented) A method of detecting a threshold temperature and hyperthermally treating tissue in an animal, said method comprising the steps of:

introducing a first fluorescent dye encapsulated in a first heat sensitive liposome into the bloodstream of an animal in a location to flow through a target site in said animal, said first fluorescent dye being releasable from said first heat sensitive liposome at a temperature of about 45°C to 49°C, and

heating said target site to a temperature to release said first fluorescent dye and fluorescing said first fluorescent dye to indicate and visualize a tissue temperature when said tissue reaches a temperature of at least 45°C, and continuing heating said target site for a time

sufficient to hyperthermally treat said tissue and kill cells in said tissue and at a temperature below a protein denaturing temperature.

Claim 11. (Cancelled)

Claim 12. (Cancelled)

13. (Previously presented) The method of claim 10, comprising heating said target site to a temperature between about 47°C and about 49°C for about 1-10 minutes.

14. (Previously presented) The method of claim 10, wherein said first liposome encapsulates a bioactive compound, and wherein said method comprises heating said first liposome to release said bioactive compound.

15. (Previously presented) The method of claim 14, wherein said bioactive compound is heat activated.

16. (Original) The method of claim 14, wherein said bioactive compound is an antiproliferative agent or an antitumor agent.

17. (Original) The method of claim 14, wherein said bioactive agent is selected from the group consisting of cisplatin, carboplatin, tetraplatin, iproplatin, adriamycin, mitomycin C, actinomycin, ansamitocin and bleomycin.

18. (Original) The method of claim 10, wherein said heat source is a laser source, a microwave source, an infrared source or an ultrasonic source.

19. (Original) The method of claim 10, wherein said heat source is a source of heated fluid and said method comprises applying said heated fluid to said target site.

20. (Previously presented) The method of claim 10, further comprising the step of

introducing a second fluorescent dye encapsulated in a second heat sensitive liposome into said bloodstream of said animal, said second fluorescent dye being releasable from said second liposome at a temperature of at least 50°C, and

visualizing and detecting said second fluorescent dye released from said second liposomes and reducing said temperature of said tissue to a temperature below 50°C in response to said detected second dye.

21. (Original) The method of claim 20, wherein said second fluorescent dye is released from said second liposome at a temperature where protein denaturization occurs, and wherein said temperature of said tissue is reduced below the protein denaturization temperature in response to said detected second fluorescent dye.

22. (Original) The method of claim 20, comprising heating said tissue in said target site to a temperature below a protein denaturization temperature of said tissue and below said release temperature of said second fluorescent dye.

23. (Previously presented) A method of hyperthermally treating tissue of an animal, said method comprising the steps of:

introducing a temperature indicating substance into the bloodstream of said animal to flow through a target site, said temperature indicating substance including a first fluorescent dye encapsulated in a first temperature sensitive liposome, said first fluorescent dye being releasable from said first liposome by heating to a temperature of about 45°C to about 49°C, and introducing a second fluorescent dye encapsulated in a second temperature sensitive liposome, said second fluorescent dye being releasable from said second liposome by heating to a temperature of at least 50°C, and

heating said target site and said first temperature sensitive liposome to a temperature sufficient to release said first liposome, and fluorescing said first fluorescent dye to indicate an effective temperature of at least 45°C for hyperthermally treating said tissue without releasing said second fluorescent dye from said second liposomes.

24. (Previously presented) The method of claim 23, comprising monitoring and detecting a fluorescence of said second fluorescent dye when released from said second temperature sensitive liposome and reducing said temperature of said tissue below a protein denaturing temperature of said tissue in response to a detection of said second fluorescent dye released from said second temperature sensitive liposome.

25. (Original) The method of claim 23, wherein said first fluorescent dye fluoresces a color different from a color of said second fluorescent dye.

26. (Original) The method of claim 23, wherein said first liposome comprises a phospholipid selected from the group consisting of dipalmitoylphosphatidyl-choline, dipalmitoylpyhosphatidyl-glycerol, and mixtures thereof.

27. (Previously presented) The method of claim 23, wherein said first liposome comprises a C_{17}-phosphatidyl-choline, wherein said second liposome releases said second fluorescent dye at a temperature of about 48°C to 49°C.

28. (Previously presented) A method of hyperthermally treating tissue of an animal, said method comprising the steps of:

introducing a temperature indicating substance into the bloodstream of said animal to flow through a target site, said temperature indicating substance including a first fluorescent dye encapsulated in a first temperature sensitive liposome, said first fluorescent dye being releasable from said first liposome by heating to a temperature of about 45°C to about 49°C, and introducing a second fluorescent encapsulated in a second temperature sensitive liposome, said second fluorescent dye being releasable from said second liposome by heating to a temperature of at least 50 C, wherein said first liposomes encapsulate a bioactive compound, and

heating said target site and said first temperature sensitive liposome to a temperature sufficient to release said first liposome, and fluorescing said first fluorescent dye to indicate an effective temperature of at least 45°C for hyperthermally treating said tissue without releasing said second fluorescent dye from said second liposomes.

29. (Original) The method of claim 28, wherein said bioactive compound is selected from the group consisting of anti-proliferative agents and anti-tumor agents.

30. (Original) The method of claim 28, wherein said bioactive compound is cis-platin.

31. (Original) The method of claim 28, wherein said bioactive compound is a photoactivated compound, and wherein said method comprises activating said photoactivated compound to kill or inhibit multiplication of cells in said target site.

32. (Previously presented) The method of claim 23, wherein said tissue is heated to a temperature of about 47°C to about 49°C.

Claim 33. (Cancelled)

34. (Original) The method of claim 23, wherein said second temperature sensitive liposomes leak or rupture at a temperature of about 50°C to 60°C.

35. (Previously presented) The method of claim 1, further comprising heating said target site to a temperature of about 47°C to about 49°C.

36. (Previously presented) The method of claim 1, further comprising the step of introducing a second liposome containing a second fluorescent dye into the bloodstream of said

animal to flow through said target site, wherein said liposome releases said dye at a temperature of about 50°C to about 60°C, and

monitoring release of said second dye from said second liposomes and reducing the temperature of said tissue in said target site to 49°C or less in response to a detection of said second dye released from said second liposomes.

37. (Previously presented) The method of claim 1, wherein said target site is in the eye.

38. (Previously presented) The method of claim 23, wherein said target site is in the eye.

39. (Previously presented) The method of claim 23, wherein said tissue in said target site is heated to a temperature below the temperature where said second dye is released from said second temperature sensitive liposome.

40. (Previously presented) The method of claim 23, further comprising monitoring and detecting release of said second fluorescent dye from said second heat sensitive liposome by fluorescing said second fluorescent dye, and

reducing the temperature of said tissue in said target site when release of said second fluorescent dye is detected.